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A REVIEW OF THE RECENT LITERATURE ON THE HEALTH ASPECTS OF GASES AS FOOD INGREDIENTS

1977

Prepared for

BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C. 20204

under

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LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike

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by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the LSRO Select Committee on GRAS Substances (SCOGS) as a part of their review of the health aspects of using these food ingredients as stipulated in the Food, Drug, and Cosmetic Act for Generally Recognized as Safe substances. Dr. Michael J. Wade prepared the report based on a comprehensive search and evaluative assessment of the current literature in accordance with the provisions of contract no. FDA 223-75-2004. Acknowledgment is made of the assistance of the LSRO staff who provided much of the background information.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

C. Jelleff Carr, Ph.D.
Director
Life Sciences Research Office

TABLE OF CONTENTS

INTRODUCTION

This report concerns the health aspects of using the gases helium, nitrous oxide, propane, n-butane, iso-butane and nitrogen as food additives. It reviews the world literature through 1976 and supplements and updates the information contained in a scientific literature review (monograph) prepared for the FDA by Informatics, Inc.[1] which summarizes the world's scientific literature up to 1973.

To assure completeness and currency as of the date of this report, information has been obtained by searches of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; and by the combined knowledge and experience of members of the LSRO staff.

There appear to be no studies in the literature involving the feeding to animals or humans of foods containing any of the six gases; consequently the studies cited in this report concerned the effects of these substances when inhaled. The gases reviewed in this study do not undergo chemical reactions under usual conditions found in mammalian tissues; and their physiological effects are normally related to their physical properties, some of which are summarized in Table I.

Studies performed at greater than atmospheric pressure are not reviewed in this report because of the difficulty in distinguishing between the effects of the inhaled gases and the effects of high pressure.

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[1]The document is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

TABLE I

Physical Properties of Gases Used in Foods

	Molecular Weight	Density[a]	Thermal Conductivity[b]	Viscosity[c]
n-butane (CH_3-CH_2-CH_2-CH_3)	58.13	2.673	35.54	83.3 (16°C)
isobutane (CH_3-$\overset{CH_3}{\overset{|}{C}}$H-$CH_3$)	58.13	2.673	36.37	75.5 (23°C)
helium (He)	4.00	0.1784	352.10	194.1
nitrogen (N_2)	28.03	1.251	60.34	175.0
nitrous oxide (N_2O)	44.01	1.977	39.30	135.0
propane (CH_3-CH_2-CH_3)	44.11	2.020	39.67	79.9 (17.9°C)
air	N.A.	1.247 (10°C)	60.34	170.8

[a] Density given in grams per liter at 0°C, 1 atmosphere pressure, except for the value for dry air which is given at 10°C and 1 atmosphere pressure.

[b] Thermal conductivity given in cal/(sec)(cm^2)(°C/cm) X 10^{-6}.

[c] Viscosity given in micropoise at 0°C, 1 atmosphere pressure, except for propane at 17.9°C, n-butane at 16°C and isobutane at 23°C.

I. HELIUM

A. BACKGROUND INFORMATION

Because of the interest in the use of helium as a substitute for nitrogen in underwater diving and space flight, there are a number of recent publications concerning exposure of man and animals to atmospheres containing helium. Helium is an inert gas and is thought not to undergo chemical reactions under physiological conditions (Parker and West, 1973). However, it has several physical properties which have physiological effects upon animals breathing helium-oxygen mixtures. Experiments concerning differential responses of animals to helium-oxygen or air environments are complicated because the thermal conductivity of helium is about six times greater than nitrogen under physiological conditions. Thus, at any given temperature below the isothermal temperature, an animal must expend more metabolic energy to maintain its normal body temperature when it is in a helium-oxygen atmosphere than when it is in air.

Sometimes a helium-oxygen atmosphere is used in an attempt to ease the breathing of patients with upper airway obstruction (Wollman and Smith, 1975). Helium-oxygen atmospheres are much less dense than air. In normal respiration in man laminar air flow occurs and the pressure required to breathe is proportional to the viscosity but not the density of the inhaled gases. However, in cases of upper airway respiratory obstruction, turbulent air flow may occur and under these conditions the pressure required to breathe is proportional to the density of the inhaled gases.

B. SHORT-TERM STUDIES

Schatte and colleagues (1973) studied the non-thermal metabolic response of rats to exposure to a helium-oxygen atmosphere. Male Holtzman rats, 8-10 weeks old (eight animals per group), were exposed to mixtures of various gases for a period of 5 days. Temperatures were maintained within 1°C of the thermal neutral temperature for each gas mixture: 31 - 33°C (for helium-oxygen) and 26 - 28°C (for nitrogen-oxygen). The animals were exposed to normoxic (sea level) and hypoxic (equivalent to 1676 m elevation) oxygen levels with either helium or nitrogen added to bring the pressure to 1 atmosphere. Under normoxic conditions the helium-exposed rats had a weight gain of 12.8 g per kg per day and the nitrogen-exposed rats had a gain of 8.7 g per kg per day. Under hypoxic conditions the helium-exposed animals had a 12.1 g per kg per day gain, while the rats exposed to nitrogen

gained only 2.9 g per kg per day. The normoxic, helium-exposed rats had a significantly lower hematocrit of 39.1 percent compared to the value of 41.6 percent in the normoxic, nitrogen-exposed rats. No significant difference in oxygen consumption was seen between rats exposed to helium or nitrogen, but the helium-exposed groups did show a significant increase in CO_2 production of 4.9 to 22.5 percent over the nitrogen-exposed rats. Experiments with labeled acetate and glucose indicated both substances were catabolized more rapidly in rats exposed to helium than to nitrogen atmospheres.

Hendrich and Weiss (1975) exposed mature male Sprague-Dawley-Holtzman rats for 30 days to an atmosphere of 79 percent helium, neon, or nitrogen and 21 percent oxygen. The parameters monitored in the experiment were body weight, food intake, plasma volume, water intake, hematocrit, anterior pituitary weight, plasma thyroid stimulating hormone, pituitary thyroid stimulating hormone and plasma protein-bound-iodine. No significant differences in any of these parameters were noted among the groups exposed to helium, nitrogen or neon.

Three-month-old rabbits were exposed for 1 week to an environment of about 23 percent oxygen and 77 percent helium or nitrogen (Hamilton *et al.*, 1970). The temperature of both environments was maintained at 26°C. Rabbits exposed to helium showed a significant increase of about 25 percent in oxygen consumption, and a tendency for increased weight gain, food consumption and heart rate. There was a significant decrease of about 10 percent in hematocrit and hemoglobin levels in the helium exposed animals. However, because the animals in the helium environment were subjected to conditions of greater environmental heat loss than the nitrogen-exposed rabbits, it is hard to distinguish between effects due to heat loss and those due to helium gas exposure.

Helium has been used in deep sea diving as an inert gas to replace nitrogen. It has only limited solubility in human tissues at atmospheric or at high pressures. In contrast, nitrogen is soluble in human tissues at high pressures and dissolved nitrogen can cause a narcosis called "rapture of the deep" at high pressures. Studies such as those of Matsuda and colleagues (1975) indicate that humans can be exposed to an environment having 5.5 atmospheres of helium for 7 days without apparent harmful effects. Helium may have some narcotic properties at 25 atmospheres of helium partial pressure (Parker and West, 1973).

C. LONG-TERM STUDIES

Harrison and Solomon (1975) found ultrastructural changes in the lung tissue of mice exposed to an 80 percent helium - 20 percent oxygen atmosphere for 160 to 350 days. The most marked histological change in the lung tissue, noted upon electron microscopic examination, was a "blebbing" that occurred in epithelial cells lining the alveolar space and endothelial cells lining the capillary lumen. Some endothelial cells had ruptured cell membranes accompanied by the deposition of a granular precipitate in the alveolar space. In endothelial cells some of the "blebs" impinged on the capillary wall, greatly diminishing its diameter. Other abnormal findings included an increased number of platelets in the capillaries and an excessive folding of the basement membranes. Similar changes were noted in the lungs of F_1 and F_2 mice reared in the helium atmosphere, while no such changes were noted in mice exposed to an 80 percent nitrogen - 20 percent oxygen atmosphere. Harrison and Solomon did not indicate how extensive the "blebbing" was nor what percentage of the animals was affected. The environmental chambers for both the helium and the nitrogen exposures were maintained at 24°C, thus the two groups of mice were exposed to different conditions of environmental heat loss.

D. SPECIAL STUDIES

The effect of breathing helium gas on heart rate and oxygen consumption in unanesthetized male albino Sprague-Dawley rats was measured by Lin and Kato (1974). A set of curves was constructed for oxygen consumption versus heart rate at different temperatures with 80 percent helium - 20 percent oxygen and air. Under conditions of identical oxygen consumption the heart rate was significantly less for animals breathing the helium-oxygen mixture than for those breathing air; and animals breathing helium-oxygen also had an enhanced positive response in heart rate to atropine and an enhanced negative response to propranolol.

Freiss *et al.* (1975) studied the isometric response of the cat gastrocnemius-soleus musculature when the animals were breathing an atmosphere of 20 percent oxygen and either 80 percent nitrogen or helium. No differences were seen in muscle response between animals breathing 80 percent helium or nitrogen upon electrical stimulation and intra-arterial infusion of the neuro-muscular junctional area with the response modifier caffeine at concentrations of 1 micromolar to 50 millimolar.

Nicholas *et al.* (1974) examined the effect of breathing an 80 percent helium - 20 percent oxygen mixture on sympathetic and cardiovascular functions. In Sprague-Dawley rats, changing the gas environment from air to helium-oxygen at 23°C significantly and reversibly depressed the pressor response to 100 nanograms of injected norepinephrine and transiently increased the heart rate but did not alter the mean heart rate. These effects were shown to be due to the different heat transfer properties of helium and nitrogen and not to any direct effect of helium on the animal. Lowering the air temperature from 26°C (thermally neutral) to 18°C had the same effect as exposure to the helium-oxygen mixture at 23°C. When rats were exposed to helium-oxygen at the thermally neutral temperature for that mixture (31°C), they showed the same pressor response and heart rate as rats breathing air at the thermally neutral temperature of 26°C. Pithed rats maintained at 37°C rectal temperature by an infrared lamp showed no differences in pressor response to injected norepinephrine, tyramine or dimethylphenylpiperazinium when they were breathing air or 80 percent helium - 20 percent oxygen. No significant differences in depressor responses elicited by subjecting rats and guinea pigs to tail down tilting emerged between animals breathing air or helium-oxygen. The response of the cat nictitating membrane to presuperior cervical ganglion stimulation did not differ significantly when cats were breathing air or helium-oxygen. There were no differences in plasma levels of circulating catecholamines when rabbits were breathing air or helium-oxygen. Similarly no differences were found in the endogenous levels or turnover of norepinephrine between rats exposed to helium-oxygen or air.

Pifare *et al.* (1970) reported that breathing mixtures containing 20 to 70 percent helium reduced the incidence of ventricular fibrillation caused by ligature of the left circumflex artery in anesthetized dogs. This effect was further investigated by Raymond *et al.* (1972). Dogs were anesthetized, subjected to ligation of the circumflex coronary artery and ventilated with 25 percent oxygen and either 75 percent helium or nitrogen for 10 minutes prior to and 2 hours following ligature. These workers also found a reduced incidence of ventricular fibrillation in the dogs breathing helium-oxygen. Prior to and following ligature, both groups of animals had similar hematocrits and levels of serum calcium, glutamic-oxaloacetate transaminase, lactic dehydrogenase and dissolved carbon dioxide and oxygen. During the course of the experiment serum potassium levels rose in the dogs breathing the nitrogen mixture; both groups showed a similar response to injected epinephrine.

In view of the above two studies suggesting helium breathing reduces the incidence of arrhythmias induced by coronary artery ligation in anesthetized dogs, Nicholas *et al.* (1975) investigated this effect in anesthetized cats, rats and mice. There was no difference in the total dose of oubain required to induce arrhythmias in rats or to flatten the

electrocardiograph tracing in mice for animals breathing air or 80 percent helium - 20 percent oxygen. The same dose of deslanoside was required to induce continuous arrhythmias and there was no difference in duration of arrhythmias induced by epinephrine in cats breathing air or helium-oxygen. Similarly there was no difference in incidence of ventricular fibrillation or frequency of arrhythmias following occlusion of the descending coronary artery between cats breathing air or helium-oxygen. These workers attempted to minimize the temperature effect of helium breathing by maintaining constant 37°C body temperature of the animals with a heated blanket and infrared lamp controlled by feedback from a rectal temperature probe.

Wang and co-workers (1970) studied the effect of helium exposure on the levels of brain gamma-aminobutyric acid. Male and female Swiss Webster strain C mice were exposed for 33 hours at 30°C to either air or 80 percent helium - 20 percent oxygen. At the end of the exposure period there was a small but significant rise of brain gamma-aminobutyric acid in the helium-exposed animals as compared to that in animals exposed to air.

The effect of breathing different gas mixtures on the volume of inspired gases has been studied in patients with obstructive emphysema and in normal volunteers. The subjects breathed a mixture of 21 percent oxygen, 0, 3, 5 or 7 percent carbon dioxide and the balance either nitrogen or helium. The normal subjects all showed a small but significant increase in inspired gas volume when breathing helium, as compared to nitrogen, at any of the carbon dioxide levels. The subjects with emphysema showed significantly increased ventilation with helium at 5 and 7 percent carbon dioxide but not at 0 or 3 percent (Grant *et al.*, 1971).

Only 25 to 30 percent of the Leghorn eggs incubated in a 79 percent helium - 21 percent oxygen atmosphere hatched compared to about 50 percent of the eggs incubated in a 79 percent nitrogen - 21 percent oxygen atmosphere (Weiss and Grimard, 1972). Both groups were incubated at 37°C. When compared with the eggs incubated in nitrogen-oxygen, those incubated in helium-oxygen had only a slightly greater egg weight loss and a slightly smaller chick hatch weight. Eggs incubated in a 79 percent argon or neon and 21 percent oxygen environment had the same hatch rate as those incubated in a nitrogen-oxygen atmosphere. Experiments using mixtures of helium and nitrogen with 21 percent oxygen showed there was a stepwise reduction in hatch ratio with decreasing concentration of nitrogen and corresponding increasing helium concentration. The authors speculate that the decrease in hatching percentage of eggs incubated in helium was not due to the high heat conductivity of helium but rather to some gas induced osmotic effect which may alter the complex moisture flux among shell, albumin, yolk and embryo.

II. NITROUS OXIDE

A. BACKGROUND INFORMATION

As with the other gases reviewed in this monograph, no studies were found that involved the feeding of foods containing nitrous oxide to humans or animals. Almost all the published work dealing with the health related effects of nitrous oxide pertains to its use as an inhaled anesthetic and analgesic.

B. ABSORPTION AND METABOLISM

Leighton and Koth (1973) reviewed the clinical pharmacology of nitrous oxide and noted inhaled nitrous oxide is rapidly absorbed from the alveoli. The solubility coefficient of nitrous oxide at 37°C was found to be 0.433 in water, 0.398 in plasma and 0.450 in red blood cells (Kozam *et al.*, 1970). In the normal human heart muscle the solubility coefficient was 0.395 for the left ventricle and 0.462 for the right ventricle. The authors suggested that this difference may be due to more fatty tissue being present in the right ventricle, nitrous oxide having a high affinity for fat.

During anesthesia with nitrous oxide, pressure and volume changes can occur in compartments of the body due to its high solubility in the tissues. When an 80 percent nitrous oxide - 20 percent oxygen mixture is inspired, nitrogen is displaced from the body and lungs and replaced with nitrous oxide. As a result of nitrous oxide's greater solubility in the tissues, the volume of nitrous oxide taken up by the tissues is 30 times greater than the nitrogen displaced (Smith, 1971). Thus, expansion and increased pressure can occur in isolated compartments of the body. For example, Saidman and Eger (1965) reported increased cerebrospinal fluid pressure in patients undergoing anesthesia with 70 - 75 percent nitrous oxide for pneumoencephalography. Increases in cerebrospinal fluid pressure were also observed in four mongrel dogs anesthetized with 75 percent nitrous oxide. Eger and Saidman (1965) investigated the effect of inhaling nitrous oxide on the bowel and intrapleural space in dogs. Sections of the bowel were tied off and injected with air. The volume of the air pocket in the isolated segment increased 100 - 200 percent after the animals breathed 70 - 80 percent nitrous oxide for 4 hours. Similarly, catheters were inserted into the pleural space of dogs and an aliquot of air was introduced. The volume of the gas pocket tripled within an hour of administration of 68 - 78 percent nitrous oxide. No such increases in volume were seen in dogs anesthetized with halothane.

Sawyer *et al.* (1974) reported that nitrous oxide is not metabolized by mini-swine, and the gas is generally assumed not to be metabolized in mammalian systems. Synthesis and metabolism of nitrous oxide do take place in microbial systems (Van Dyke, 1972; Webster and Hinn, 1966), but no reports giving evidence that nitrous oxide undergoes metabolism in mammals have been found in the course of this review. However, Cohen (1973) speculates such evidence is lacking only because of the non-availability of a suitable radioactive form of nitrous oxide.

C. ACUTE TOXICITY

There are accounts in the literature of deaths associated with the use of nitrous oxide in dental anesthesia. For example, Bourne (1973) described two fatalities in which dental patients were anesthetized with mixtures of nitrous oxide and oxygen in conjunction with either halothane or injected methohexitone. In both cases the victims were healthy young women who suddenly collapsed during anesthesia; there was no asphyxia, oxygen shortage, evidence of underlying disease or error in administration of anesthesia. Ruben (1972) reported that in 15 years of dental use of nitrous oxide in Denmark an estimated 3 million analgesias had been administered. There were no official records of any complications resulting from dental use of nitrous oxide in Denmark and attempts by Ruben to trace unconfirmed reports of complications were unsuccessful. However, of the 3,000 dental practitioners in Denmark in 1970, Ruben reported that three of them were addicted to nitrous oxide.

D. SHORT-TERM STUDIES

Damage and destruction of sperm cells occurred in 125 male LEW/f Mai rats exposed to 20 percent nitrous oxide either continuously or intermittently for 8 hours a day (Kripke *et al.*, 1976). Damage to spermatocytes, spermatids and spermatozoa could first be detected after exposure for 2 days to nitrous oxide. After 14 days of exposure, the changes became more pronounced and were seen in all the rats examined from either the continuous or intermittent exposure groups. There was a reduction in the number of spermatocytes and some cells showed disruption of the normal sperm structure. Some multinucleated giant cells were seen. Supporting cells such as the interstitial cells of Leydig and Serotoli cells were apparently not affected. Recovery occurred in some animals continuously exposed for 32 days and then removed to room air. Repair of cell ultrastructure and normal maturation and development of

spermatozoa were seen. However, some animals still showed depressed spermatogenesis 10 days after exposure. Compared to 75 control animals breathing room air, the nitrous oxide-exposed group had about a 50 percent decreased testicular weight following 28 days of nitrous oxide treatment. By contrast, after 32 days of nitrous oxide treatment followed by 6 days in air, the control and exposed groups had similar testicular weights. Other than damage to sperm cells, Kripke *et al.* (1976) mentioned no toxic effects of nitrous oxide.

E. SENSITIVITY

Malignant hyperexia can occur in susceptible individuals who are exposed to anesthetics, including nitrous oxide. The tendency towards this trait is inherited as an autosomal dominant. The condition is frequently fatal and characterized by a rapid increase in body temperature and muscular rigidity. Ryan and Appleyard (1975) have described a family study of persons prone to this disorder. Multiple use of anesthetics made it difficult to definitively identify which substance triggered the condition but a male member of the susceptible family developed the condition and died upon exposure to nitrous oxide during anesthesia. Subsequent follow up revealed the deceased's twin daughters had recently undergone anesthesia; one of the twins developed malignant hyperexia after nitrous oxide exposure, while the other was anesthetized without nitrous oxide and suffered no ill effects.

F. SPECIAL STUDIES

1. Studies on teratogenesis, mutagenesis and cell division

Exposure of mated female rats to an atmosphere of 1,000 ppm nitrous oxide continuously for 12 to 19 days caused a significant decrease in the number of implantations per rat and a significant increase in the fetal death rate (Corbett *et al.*, 1973). In the rat, lethal effects manifested shortly after conception may result in total resorption of the conceptus by day 20, leaving no evidence of pregnancy; however, this results in a lowering of the ratio of implantations per rat. A significant increase in fetal death rate was seen in rats exposed 8 hours daily for 10 to 19 days to 1,000 ppm nitrous oxide. Exposure to 100 ppm nitrous oxide for 8 hours daily caused a significant increase in fetal death in only one of two groups of exposed rats.

Brinkley and Rao (1973) examined the effects of nitrous oxide on the mitotic apparatus and chromosome movement in HeLa cells. Cells which had been previously partially synchronized by thymidine blockade were exposed to nitrous oxide at 1.42 kg per cm^2 for up to 9 hours, then either fixed or transferred to an incubator and allowed to recover. Cells fixed immediately following nitrous oxide treatment showed mitotic arrest with abnormal distribution of chromosomes on the mitotic spindle. Some cells showed abnormal mitochondria with distorted vesicular cristae. Cells transferred to the incubator at atmospheric pressure following nitrous oxide exposure underwent metaphase but a high incidence of multipolar spindles was observed. With nitrous oxide-exposure-times of from 4 to 36 hours, a direct relationship between length of exposure and number of multipolar spindles was observed. No abnormal mitochondria were seen in cells allowed to recover from nitrous oxide blockade. The authors stated that their previous work involving exposure of HeLa cells to nitrogen atmospheres ruled out anoxia as a cause of the mitotic blockade.

Sex-linked lethal mutations were reported to be induced in male Drosophilla melanogaster exposed to nitrous oxide (Garret and Fuerst, 1974).

2. Cardiovascular effects

Eisle and Smith (1972) studied the cardiovascular effects of 40 percent nitrous oxide inhalation on 10 volunteers. The subjects were first familiarized to inhalation of the gas by daily 45-minute administrations of 40 percent nitrous oxide for 3 to 4 weeks. Compared to control subjects breathing 40 percent nitrogen - 60 percent oxygen, there was a reduction in heart rate, magnitude of the balistocardiogram wave, cardiac output and forearm blood flow during a 40-minute period of inhalation of 40 percent nitrous oxide - 60 percent oxygen. Normal blood pressure was maintained and there was a slight increase in central venous pressure. During exposure most of the subjects experienced alternating periods of "going under" and recovery. There were reports of analgesia and hyperacusis but no indications of anxiety or excitement.

No direct action of nitrous oxide on rat myocardium was found by Goldberg *et al.* (1972). Isolated rat ventricular preparations were equilibrated with a mixture of 95 percent oxygen and 5 percent carbon dioxide and then exposed to 25, 50 and 75 percent nitrogen or nitrous oxide for 15 minutes. No significant differences were found between nitrous oxide or nitrogen in depression of the peak developed tension and maximum rate of tension development. No changes were caused by the gases in time to reach peak isometric tension and time for tension to decay to 90 percent of maximum. Goldberg *et al.* (1972) concluded that nitrous oxide produces no intrinsic myocardial depressant or stimulant effect, and any change in

myocardial contractility associated with nitrous oxide administration should be related to changes in extrinsic factors like sympathetic activation or oxygenation of the myocardium.

3. Biochemical studies

In response to earlier studies suggesting that anesthesia may suppress immunologic defense against infection, Cullen (1974) examined the effect of nitrous oxide on leukocytes. Suspended human leukocytes were equilibrated with an atmosphere of 80 percent nitrous oxide in oxygen. After 45 minutes of incubation, there was a small, insignificant decrease in ability of the cells to phagocytize latex spheres and to reduce nitroblue tetrazolium as compared to control suspensions incubated in air.

Hallen and Johansson (1975) examined the action of inhaled nitrous oxide on the activities of nine liver microsomal cytochrome p-450 dependent enzymes. Rats were anesthetized in chambers through which a three to one mixture of nitrous oxide to oxygen was circulated. After 1 hour, the rats were immediately killed and their livers removed. Following isolation of the microsomal fraction a number of the cytochrome p-450 dependent enzyme activities were assayed. Compared to control animals exposed to air, there were no differences in any of the nine enzyme activities measured.

Nitrous oxide is capable of supporting combustion and under anaerobic conditions, can oxidize the vitamin B_{12} cofactor of the enzyme dioldehydrase (sic propanediol dehydratase, EC 4.2.1.28) (Price, 1975; Schrauzer, 1973).

4. Behavioral studies

On the basis of studies showing that nitrous oxide analgesia in mice could be blocked by the opiate antagonists haloxone hydrochloride and haltrexone or by chronic morphine administration, Berkowitz *et al.* (1976) speculated nitrous oxide analgesia could be due to release or potentiation of an endogenous, naturally-occurring substance with opiate-like action.

Administration of 10 to 30 percent nitrous oxide in oxygen caused diminution of the auditory evoked response and reaction time in 12 volunteer subjects (Lader and Jarvis, 1974).

Brodsky and Zuniga (1975) characterized nitrous oxide as a psychotogenetic agent. They describe the case of a 32-year-old male dentist with no prior history of confusion or psychotic symptoms; but when confronted with a period of increased stress in his life, increasingly used nitrous oxide to "help him relax" and place him in a "somnolent state of bliss." After he began inhaling 45 - 65 percent nitrous oxide 4 or 5 times

daily for 3 months, the subject began to have paranoid delusions and was subsequently hospitalized.

5. Chemical studies

Goldstein *et al.* (1976) observed an increase in levels of nitrogen dioxide and nitric oxide (NO) in operating room atmospheres while surgery was performed using nitrous oxide anesthesia. The increased levels of nitric oxide and nitrogen dioxide peaked during operation of energy-releasing devices such as portable x-ray machines and electric cauteries suggesting oxidation of nitrous oxide by these devices. No instances of oxidation of nitrous oxide associated with its use in food were uncovered. However, Webster (1966) has reported the decomposition of nitrous oxide to nitrogen and water when it was used as a propellant in a container in the presence of water and oxygen at pH 3.3.

The United States Pharmacopeia (1973) lists 0.0001 percent as the maximum amount of nitric oxide or nitrogen dioxide permissible in nitrous oxide intended for analgesic or anesthetic use. Methemoglobinemia and pulmonary edema occur after acute exposure to nitric oxide and nitrogen dioxide (Malatinsky *et al.*, 1973). Nunn (1967) reported on an incident in England where several people were poisoned, one fatally, due to contamination with nitric oxide and nitrogen dioxide of a cylinder of nitrous oxide used for anesthesia.

III. PROPANE AND BUTANE

A. BACKGROUND INFORMATION

Few recent studies dealing with the health aspects of propane or butane were found; none concerned their possible effects when used as a gas in food. Because most of these studies considered both butane and propane, and because of the chemical similarities of these hydrocarbons, they will be considered together. Both the linear and branched chain forms of butane (n-butane and isobutane) will be reviewed in this section.

B. ABSORPTION

The absorption of inhaled propane or butane was measured in humans breathing air containing 100 ppm propane, n-butane or isobutane for 20 minutes. After 20 minutes it was found that 12 percent of an inhaled dose of 100 ppm propane and 14 percent of either 100 ppm n-butane or isobutane would be absorbed (Wagner, 1975). Measurements were only made at the single time point of 20 minutes. Presumably there would have been a higher percentage uptake of the inhaled hydrocarbon when the subjects initially began breathing the gas.

C. ACUTE TOXICITY

Twenty fatal cases of propane gas inhalation in the greater Tokyo area were investigated by Ikoma (1972). Of the deaths, 19 were suicides and the 20th an industrial accident. The deaths were associated with the use of domestic fuel; gas (colloquially called propane gas) consisting of a mixture of propane, propylene, isobutane, n-butane and small amounts of other gases with propane and propylene being the major constituents. The composition of the mixture varied depending on the supplier. Propane and propylene were identified in the blood, urine and cerebrospinal fluid of the victims. Also noted upon postmortem examination were cyanosis in the lips and fingernail beds, congestion of blood vessels in the trachea, cerebral and pulmonary edema and a characteristic "propane fuel odor" during thoractomy. Cause of the deaths was listed as aphyxiation due to lack of oxygen.

D. SPECIAL STUDIES

In the mouse, propane and isobutane were found to sensitize the heart to epinephrine-induced arrhythmias (Aviado and Belej, 1974). Male Swiss mice were anesthetized with sodium pentobarbital and exposed to an atmosphere of 10 or 20 percent propane or 20 percent isobutane for 20 minutes. Another group was similarly treated, but also injected with 6 μg per kg of epinephrine two minutes after the start of isobutane or propane exposure. No electrocardiogram abnormalities were found in mice treated with either one of the gases or epinephrine alone. However, treatment with 10 or 20 percent propane or 20 percent isobutane and epinephrine resulted in incidents of sinus tachycardia, inverted T wave, second degree block and other arrhythmias. Propane and isobutane have also been shown to sensitize unanesthetized dogs to epinephrine-induced arrhythmias (Reinhardt *et al.*, 1971).

Freidman *et al.* (1974) studied the circulatory and pulmonary effects of inhaled isobutane on 55 male Mendel-Osborne rats. The animals were anesthetized by intraperitoneal injection of diallylbarbituric acid (60 mg per kg) and urethane (240 mg per kg). The rats were then subjected to increasing concentrations of iso-butane. Apnea occurred when the gas concentration reached 27 percent with cessation of heart beat following in a few minutes.

Inhalation of up to 20 percent propane for 5 minutes did not influence cardiac rhythm, heart rate, contractility, aortic blood pressure, left atrial pressure or pulmonary arterial pressure in rhesus monkeys anesthetized by intravenous injection of 30 mg per kg sodium pentobarbitol (Belej *et al.*, 1974). However, in the same study it was found that inhalation of 10 percent isobutane for 5 minutes caused a 10 percent increase in heart rate and a 25 percent decrease in myocardial force as measured by a strain gauge attached to the left ventricular surface. In contrast to what was earlier observed in the mouse, Belej *et al.* found that inhalation of isobutane at 5 to 10 percent concentrations did cause unspecified cardiac arrhythmias in the monkey.

Further studies by Aviado and Smith (1975) on the sodium pentobarbital anesthetized monkey demonstrated that inhalation of 20 percent propane for 5 minutes resulted in a 10 percent increase in pulmonary resistance and a 20 percent reduction in inspired air volume. After inhalation of 10 percent isobutane for 5 minutes there was a 20 percent increase in pulmonary resistance, a 13 percent decrease in inspired air volume, and a 22 percent fall in aortic blood pressure.

On the basis of the above studies, Aviado (1975) has proposed that propane and isobutane be classified as aerosol propellants of intermediate toxicity.

Watanabe and Takesue (1972) reported that bubbling propane, n-butane or isobutane through a solution of egg white lysozyme inhibited the enzymes' ability to lyse Micrococcus lysodeikticus cells. The inhibition could be partially reversed by bubbling air through the enzyme solution. No inhibition occurred when the gases were bubbled through the enzyme-substrate solution after the start of enzymatic lysis, but only when the gas was bubbled through the enzyme solution prior to its addition to the bacterial cells. A competitive inhibition of lysozyme by the three gases was indicated by Lineweaver-Burke plots.

IV. NITROGEN

The atmosphere consists of about 80 percent nitrogen and this gas is nontoxic under most conditions. Suffocation by nitrogen can occur under conditions of poor ventilation if its concentration rises and too much oxygen is displaced. Nitrogen gas is not necessary for human life and some space cabin atmospheres consist of essentially pure oxygen. Nitrogen narcosis occurs when the gas is breathed under high pressure (greater than about 4 atmospheres) such as in diving (Peirce, 1974), and a condition called the "bends" can occur when divers breathing compressed air ascend to the surface too rapidly. This condition is caused by pockets of nitrogen forming in the tissues. As the diver ascends and the pressure becomes less, nitrogen which was dissolved in the tissues goes out of solution and forms nitrogen pockets and bubbles. Nitrogen is not thought to be metabolized in mammalian systems; it is, however, reduced to ammonia by soil microorganisms. No studies related to the use of nitrogen as a gas in food, or any toxic effects of nitrogen under normal physiological conditions has been found in the course of these review studies.

V. REFERENCES CITED

Aviado, D. and D.G. Smith. 1975. Toxicity of aerosol propellants in the respiratory and circulatory systems. VIII. Respiration and circulation in primates. Toxicology 3: 241-252.

Aviado, D.M. 1975. Toxicity of aerosol propellants in the respiratory and circulatory systems. X. Proposed classification. Toxicology 3: 321-332.

Aviado, D.M. and M.A. Belej. 1974. Toxicity of aerosol propellants on the respiratory and circulatory systems. I. Cardiac arrhythmia in the mouse. Toxicology 2: 31-42.

Belej, M.A., D.G. Smith and D.M. Aviado. 1974. Toxicity of aerosol propellants in the respiratory and circulatory systems. IV. Cardiotoxicity in the monkey. Toxicology 2: 381-395.

Bennett, P.B. 1969. Inert gas narcosis. Pages 155-182 in P.B. Bennett and D.H. Elliott, eds. The physiology and medicine of diving and compressed air work. The Williams and Wilkins Company, Baltimore, Md.

Berkowitz, B.A., S.H. Ngai and A.D. Finck. 1976. Nitrous oxide "analgesia": resemblance to opiate action. Science 194: 967-968.

Bourne, J.G. 1973. Deaths associated with dental anaesthetics. Lancet 1: 35.

Brinkley, B.R. and P.N. Rao. 1973. Nitrous oxide: effects on the mitotic apparatus and chromosome movement in HeLa cells. J. Cell Biol. 58: 96-106.

Brodsky, L. and J. Zuniga. 1975. Nitrous oxide: a psychotogenic agent. Compr. Psychiatry 16: 185-188.

Cohen, E.N. 1971. Metabolism of the volatile anesthetics. Anesthesiology 35: 193-202.

Corbett, T.H., R.G. Cornell, J.L. Endres and R.I. Millard. 1973. Effects of low concentrations of nitrous oxide on rat pregnancy. Anesthesiology 39: 299-301.

Cullen, B.F. 1974. The effect of halothane and nitrous oxide on phagocytosis and human leukocyte metabolism. Anesth. Analg. 53: 531-535.

Eger, E.I. and L.J. Saidman. 1965. Hazards of nitrous oxide anesthesia in bowel obstruction and pneumothorax. Anesthesiology 26: 61-66.

Eisele, J.H. and N.T. Smith. 1972. Cardiovascular effects of 40 percent nitrous oxide in man. Anesth. Analg. 51: 956-963.

Freidman, S.A., M. Cammarato and D.M. Aviado. 1973. Toxicity of aerosol propellants on the respiratory and circulatory systems. II. Respiratory and bronchopulmonary effects in the rat. Toxicology 1: 345-355.

Friess, S.R., R.C. Durant, W.V. Hudak and R.D. Boyer. 1975. Effects of moderate pressure He-0_2 saturation and response modifiers on neuromuscular function. Undersea Biomed. Res. 2: 35-41.

Garrett, S. and R. Fuerst. 1974. Sex-linked mutations in Drosophila after exposure to various mixtures of gas atmospheres. Environ. Res. 7: 286-293.

Goldberg, A.H., Y.Z. Sohn and W.P.C. Phear. 1972. Direct myocardial effects of nitrous oxide. Anesthesiology 37: 373-380.

Goldstein, B.D., J.G. Giuffrida, J. Paz, E.D. Palmes and E.F. Ferrand. 1976. Atmospheric derivatives of anaesthetic gases as a possible hazard to operating-room personnel. Lancet 2: 235-237.

Grant, J.L., E. Lucier and M.L. Mahnke. 1971. Estimation of respiratory drive with carbon dioxide and helium. Ann. Intern. Med. 74: 62-66.

Hallen, B. and G. Johansson. 1975. Inhalation anesthetics and cytochrome P-450-dependent reactions in rat liver microsomes. Anesthesiology 43: 34-40.

Hamilton, R.W., Jr., G.F. Doebbler and H.R. Schreiner. 1970. Biological evaluation of various spacecraft cabin atmospheres. I. Space Life Sci. 2: 307-334.

Harrison, G.A. and J.D. Solomon. 1975. Ultrastructural alterations observed in mouse lung after prolonged He/0_2 exposure. Aviat. Space Environ. Med. 46: 21-25.

Hendrich, C.E. and H.S. Weiss. 1975. Pituitary-thyroid function of rats in hypobaric oxygen-inert gas environments. Am. J. Physiol. 228: 1210-1216.

Ikoma, T. 1972. [A study on sudden death from inhalation of propane gas for household use.] Nichidai Igaku Zasshi 31(2): 71-81. (In Japanese)

Kozam, R.L., S.M. Landau, J.M. Cubina and D.S. Lukas. 1970. Solubility of nitrous oxide in biologic fluid and myocardium. J. Appl. Physiol. 29: 593-597.

Kripke, B.J., A.D. Kelman, N.K. Shah, K. Balogh and A.H. Handler. 1976. Testicular reaction to prolonged exposure to nitrous oxide. Anesthesiology 44: 104-113.

Lader, M.H. and M.J. Jarvis. 1974. Effects of nitrous oxide on the evoked response. Act. Nerv. Super. 16: 50-52.

Leighton, K.M. and B. Koth. 1973. Some aspects of the clinical pharmacology of nitrous oxide. Can. Anaesth. Soc. J. 20: 94-103.

Lin, Y.C. and E.N. Kato. 1974. Effects of helium gas on heart rate and oxygen consumption in unanesthetized rats. Undersea Biomed. Res. 1: 281-290.

Malatinský, J., T. Kadlic and V. Kováčik. 1973. Acute poisoning by higher nitrogen oxides. Anesth. Analg. 52: 94-99.

Matsuda, M., H. Nakayama, A. Itoh, N. Kirigaya, F.K. Kurata, R.H. Strauss and S.K. Hong. 1975. Physiology of man during a 10-day dry heliox saturation dive (SEATOPIA) to 7 ATA. I. Cardiovascular and thermoregulatory functions. Undersea Biomed. Res. 2: 101-118.

Matsuda, M., H. Nakayama, F.K. Kurata, J.R. Claybaugh and S.K. Hong. 1975. Physiology of man during a 10-day dry heliox saturation dive (SEATOPIA) to 7 ATA. II. Urinary water, electrolytes, ADH and aldosterone. Undersea Biomed. Res. 2: 119-131.

Nicholas, T.E., J. Hart and P.A. Kim. 1974. Effect of breathing helium on sympathetic nervous and cardiovascular functions. Undersea Biomed. Res. 1: 271-280.

Nicholas, T.E., J.L. Hart and P.A. Kim. 1975. Inability of helium to influence the occurrence of arrhythmias in cats, rats and mice. Undersea Biomed. Res. 2: 28-34.

Nunn, J.F. 1969. Higher oxides of nitrogen as a contaminant of nitrous oxide. Pages 70-77 in V.L. Brechner and C.M. Ballinger, eds. Pharmacology of anesthetic agents and their measurement. (Proceedings of the 1967 Biennial Western Conference on Anesthesiology) University of Utah Press, Salt Lake City, Utah.

Parker, J.F. and V.R. West, eds. 1973. The inert gases. Pages 52-54, 56-58 in Bioastronautics data book, 2nd ed. NASA SP-3006. U.S. Government Printing Office, Washington, D.C.

Peirce, E.C., II. 1974. Problems of inert gases. N.Y. State J. Med. 74: 813-819.

Pifarré, R., T.K. Raghunath, R.M. Vanecko, F.S. Chua, J.U. Balis and W.E. Neville. 1970. Effect of oxygen and helium mixtures on ventricular fibrillation. J. Thorac. Cardiovasc. Surg. 60: 648-652.

Raymond, L., R.B. Weiskopf, M.J. Halsey, A. Goldfien, E.I. Eger, III, and J.W. Severinghaus. 1972. Possible mechanism for the antiarrhythmic effect of helium in anesthetized dogs. Science 176: 1250-1252.

Reinhardt, C.F., A. Azar, M.E. Maxfield, P.E. Smith, Jr. and L.S. Mullin. 1971. Cardiac arrhythmias and aerosol "sniffing." Arch. Environ. Health 22: 265-279.

Ruben, H. 1972. Nitrous oxide analgesia in dentistry: its use during 15 years in Denmark. Br. Dent. J. 132: 195-196.

Ryan, D.W. and T.N. Appleyard. 1975. Malignant hyperpyrexia: investigation of a family after a fatal case. Br. J. Anaesth. 47: 1001-1004.

Saidman, L.J. and E.I. Eger, II. 1965. Change in cerebrospinal fluid pressure during pneumoencephalography under nitrous oxide anesthesia. Anesthesiology 26: 67-72.

Sawyer, D.C., E.I. Eger, II, S.H. Bahlman, M.J. Cullen and D.M. Impelman. 1972. Metabolism of inhalation anesthetics. Pages 238-244 in B.R. Fink, ed. Cellular Biology and Toxicity of Anesthetics. The Williams and Wilkins Company, Baltimore, Md.

Schatte, C.L., J.P. Jordan, R.W. Phillips, D.P. Clarkson and J.B. Simmons, II. 1973. Nonthermal metabolic response of rats to He-O_2, N_2-O_2 and Ar-O_2 at 1 atm. Am. J. Physiol. 225: 553-558.

Schrauzer, G.N., J.A. Seck and R.J. Holland. 1973. Dioldehydrase: new observations concerning the effect of oxygen, nitrous oxide and carbon monoxide. Z. Naturforsch. Teil. C Biochem. Biophys. Biol. Virol. 28: 1-3.

Smith, W.D.A. 1971. Uptake, distribution and elimination. Pharmacology of nitrous oxide. Int. Anesthesiol. Clin. 9: 91-123.

United States Pharmacopeial Convention, Inc. 1975. Nitrous oxide. Page 344 in The United States Pharmacopeia, 19th rev. Rockville, Md.

Van Dyke, R.A. 1972. Biotransformation. Pages 351-355 in M.B. Chenoweth, ed. Modern inhalation anesthetics. Springer-Verlag, New York, N.Y.

Wagner, H.M. 1974. Retention einiger Kohlenwasserstoffe bei der Inhalation. Schriftenr. Ver. Wasser-, Boden-Lufthyg., Berlin-Dahlem. 41: 225-229.

Wang, D.-M., R.L. Reinke and R.H. Wilson. 1970. Comparison of the effect of length of exposure to high pressure helium-oxygen on brain gamma-amino-butyric acid levels in male and female mice. Aerosp. Med. 41: 526-529.

Washburn, E.W., Editor-in-Chief. 1928. International critical tables of numerical data, physics, chemistry and technology. 1st ed., vol. 3, prepared by the National Research Council, U.S.A. McGraw-Hill Book Company, Inc., New York, N.Y.

Washburn, E.W., Editor-in-Chief. 1929. International critical tables of numerical data, physics, chemistry and technology. 1st ed., vol. 5, prepared by the National Research Council, U.S.A. McGraw-Hill Book Company, Inc., New York, N.Y.

Watanabe, K. and S. Takesue. 1972. Inhibitory effect of some gaseous hydrocarbons on the cell-lysis of Micrococcus lysodeiktus by egg-white lecithin. Agric. Biol. Chem. 36: 825-830.

Weast, R.C., ed. 1971. Handbook of chemistry and physics, 52nd ed., 1971-1972. The Chemical Rubber Co., Cleveland, Ohio.

Webster, R.C. 1966. Compressed Gases. Pages 191-194 in A. Herzka, ed. International encyclopaedia of pressurized packaging (aerosols). Pergamon Press, New York, N.Y.

Webster, R.C. and J.S. Hinn. 1966. Foods. Page 413 in A. Herzka, ed. International encyclopaedia of pressurized packaging (aerosols) Pergamon Press, New York, N.Y.

Weiss, H.S. and M. Grimard. 1972. Inert gas effects on embryonic development. J. Appl. Physiol. 33: 375-380.

Wollman, H. and T.C. Smith. 1975. The therapeutic gases: oxygen, carbon dioxide, and helium. Pages 881-899 in L.S. Goodman and A. Gilman, eds. The pharmacological basis of therapeutics, 5th ed. Pergamon Press, New York, N.Y.